Progression of Nodular Poorly Differentiated Lymphocytic Lymphoma to Burkitt’s-Like Lymphoma

By David M. Mintzer, Michael Andreeff, Daniel A. Filippa, Suresh C. Jhanwar, R.S.K. Chaganti, and Benjamin Koziner

Histologic conversion of nodular lymphomas to more aggressive patterns has been well described and occurs in 15% to 40% of cases. Most conversions have involved changes from nodular to diffuse patterns and often from small to larger cell types. Conversions to undifferentiated lymphomas, but not specifically to the Burkitt’s type, have infrequently been described. This report describes three cases of nodular poorly differentiated lymphocytic lymphoma that converted to a Burkitt’s-like lymphoma. Conversion was associated with short survivals, increasing lactate dehydrogenase (LDH) levels, and changes in DNA stemline, RNA content, and S-phase values, as determined by flow cytometry. Karyotypes in two cases revealed a t(14;18). Surface immunoglobulin could be demonstrated on transformed Burkitt’s-like cells from lymph node in one case, but in none of the cases on cells from bone marrow.

The classifications of non-Hodgkin’s lymphoma attempt to distinguish between “favorable” and “unfavorable” histologies. In the modified Rappaport classification, the lymphomas are divided into nodular or diffuse on the basis of lymph node architecture, and into lymphocytic or “histiocytic” on the basis of individual cellular morphology. Favorable lymphomas include diffuse well-differentiated lymphocytic lymphoma, nodular poorly differentiated lymphocytic lymphoma, and nodular mixed lymphoma. Unfavorable histologies include nodular histiocytic and all other diffuse lymphomas. It is well recognized, however, that the favorable lymphomas may convert to more aggressive ones and that such conversion may be associated with shortened survivals. This typically involves the progression from nodular to diffuse patterns and often from a predominance of small cells (lymphocytic) to large cells (“histiocytic”). Conversions of nodular lymphocytic lymphoma to undifferentiated lymphoma have been infrequently described, and conversion to a Burkitt’s-like lymphoma has not been specifically reported. This report describes three cases of nodular poorly differentiated lymphocytic lymphoma (N-PDLL) that converted to a Burkitt’s-like lymphoma, with characterization of clinical, cytologic, cytogenetic, and kinetic features.

Case Reports

Case 1

A 52-year-old woman developed inguinal adenopathy in 1978. Biopsy revealed N-PDLL, and staging work-up revealed marrow involvement. She received the NHL-4 protocol (thiotepa, vincristine, prednisone, chlorambucil, cyclophosphamide, and doxorubicin) in June 1980. The patient was in complete remission and chemotherapy was stopped. In June 1981, she developed abdominal recurrence and was treated with radiation therapy. In October 1981, axillary adenopathy developed, and biopsy again revealed N-PDLL. A trial of interferon therapy was stopped because of cytopenia. She received no therapy until July 1982, when rapidly progressive supraclavicular and cervical adenopathy was noted. Lactate dehydrogenase (LDH) was 471 U/L. Marrow aspirate contained typical L3 cells. Biopsy of an axillary mass revealed a diffuse undifferentiated lymphoma, resembling Burkitt’s, replacing two thirds of the node, with the remaining portion showing residual N-PDLL. The patient received the NHL-7 protocol (methyl-GAG, bleomycin, cyclophosphamide, and prednisone), but 1 month later, she developed progressive systemic and meningeal disease. LDH was 657 U/L. She received daunomycin, cytosine arabinoside, and methotrexate (systemic and intrathecal) but died one month later.

Case 2

A 44-year-old man presented in 1975 with axillary adenopathy. Biopsy revealed N-PDLL, and staging work-up revealed marrow involvement. He received cyclophosphamide, chlorambucil, vincristine, prednisone, and radiation therapy to a mantle field. After February 1976, therapy was continued with chlorambucil alone. He remained in remission until 1980, when axillary adenopathy recurred, and he was treated with local radiation therapy and continued chlorambucil. In March 1983, a WBC of 27,000/µL with 30% blasts was noted. Marrow aspirate revealed infiltration with immature lymphoid cells with pale blue cytoplasm and immature chromatin. LDH was 6,762 U/L. The patient received vincristine, prednisone, cyclophosphamide, and doxorubicin over five weeks, with apparent response. Two weeks later, however, he developed abdominal pain and distention. LDH was 1,339 U/L. Repeat marrow revealed infiltration with typical L3 cells, clearly different from the cells noted eight weeks earlier. Computerized tomography showed extensive mesenteric and retroperitoneal lymphoma. He received daunomycin, cytosine arabinoside, and 6-thioguanine with a rapid response, but four weeks following treatment he developed fulminant recurrence in the abdomen, lymph nodes, bone marrow, and blood. He died in June 1983.

Case 3

A 50-year-old man developed generalized lymphadenopathy in May 1979. In October 1979, biopsy of a supraclavicular lymph node...
typic analysis was performed on Q-banded preparations. Flow cytometry for determination of cell cycle distribution, DNA stemline, and RNA content was performed as previously described. The DNA stemline of normal cells was used as reference and designated 2.0C. RNA index was calculated as the relative RNA content of the G0/1 cells of the sample compared to normal control peripheral blood lymphocytes.

RESULTS

Patient characteristics are summarized in Table 1. Bone marrow aspirates from each patient at the time of conversion revealed a predominance of L3 cells (Fig 2). In case 1, lymph node biopsy at conversion revealed undifferentiated lymphoma with Burkitt’s-like features replacing two thirds of the lymph node, with residual areas of N-PDLL (Fig 1). Lymph node biopsies were not performed at conversion in the other two patients.

Surface marker analysis performed prior to Burkitt’s-like transformation in each case demonstrated a B cell proliferation (Table 2), with a predominance of IgGA (case 1), IgGk (case 2), and IgM, light chain undetermined (case 3). Following conversion in case 1, immunofluorescence of lymph node revealed persistence of IgGk expression, although there was a decrease in EAC-forming cells. In none of the cases was surface immunoglobulin (slg) detectable on cells from involved marrow.

DNA flow cytometry revealed a change in DNA stemline in each case following conversion (Table 2): in cases 1 and 3 from 2.0 to 2.5 and 2.2, respectively, and in case 2 from 2.1 to 1.9 (Fig 3). S-phase values were elevated to 20%, 26%, and 15% following conversion. Values for RNA index were low prior to and elevated following transformation.

Karyotypic analysis of involved bone marrow at transformation revealed analyzable metaphases in two cases. In case 1, of 27 cells analyzed, three (11%) were normal and 24 (89%) were abnormal. The latter comprised a pseudodiploid clone that exhibited the translocation t(14;18)(q32;q21) and the following additional abnormalities. One chromosome 1 was replaced by an i(Iq) and the short arm of the other was abnormal; its derivation could not be determined by the banding pattern. The long arm of one chromosome 3 was replaced by a slightly longer segment, whose

MATERIALS AND METHODS

Tetrachrome-stained smears of bone marrow were reviewed by three different investigators. Lymphocyte preparation and immunofluorescent staining were performed as previously described. For cytogenetic studies, aspirated bone marrow was cultured for 24 hours in RPMI 1640 medium supplemented with fetal bovine serum and antibiotics. Chromosome preparations were made following conventional methods, using 0.075 mol potassium chloride as the hypotonic solution and 3:1 methanol:acetic acid as fixative. Karyo-
Fig 3. DNA-RNA histograms of bone marrow cells from patient 2: cells with high RNA content (RNA index 18.4) have high proliferation ($S = 26\%$) and an abnormal low DNA stemline (1.9C). This is shown in the right-hand panel, when the sample was measured at maximal sensitivity for DNA. This was corroborated by karyotyping (44 chromosomes).

derivation could not be determined by its banding pattern. Chromosome 5 was present in a single copy. The entire short arm of chromosome 9 was replaced by the entire short arms of chromosome 1. One chromosome 12 was replaced by an i(12q). Finally, one short acrocentric chromosome, the site of an E-group chromosome, was present. Nonclonal abnormal chromosomes replacing normal chromosomes were encountered in occasional cells. The karyotype of clonal cells, according to ISCN (1978), was: 46, XX, −I, −1, −3, −5, −9, −12, −13, −14, −18, +i(1q)+der(1), t(1;?)p(?)i(1q)+der(3), t(3;?)q12?+der(9), t(1;9)(p11;p11)+i(12q), t(14;18)(q32;q21), +2mar (Fig 4A).

In case 2, of 30 cells analyzed, 12 (40%) were normal and 18 (60%) were abnormal. The latter comprised a hypodiploid clone that exhibited the translocation t(14;18)(q32;q21) and the following additional abnormalities. A segment of unknown derivation was translocated to chromosome 6 at band q62q1. The entire short arm of chromosome 8 was deleted. Chromosome 10 had a deletion in the long arm of the segment distal to band q24. Three marker chromosomes, whose derivation could not be determined by their banding pattern, were present in each clonal cell.

Table 2. Laboratory Results

<table>
<thead>
<tr>
<th>Case</th>
<th>Date</th>
<th>Specimen</th>
<th>Morph</th>
<th>Percent L3 Cells</th>
<th>S Phase DNA Stemline</th>
<th>RNA Index</th>
<th>Percent Cells With Abnormal DNA Stemline</th>
<th>SRBC</th>
<th>EAC</th>
<th>Fc</th>
<th>$\mu$</th>
<th>$\gamma$</th>
<th>$\kappa$</th>
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<td>11.1</td>
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<td>20</td>
<td>27</td>
<td>0</td>
<td>18</td>
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<td>3</td>
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<td>0</td>
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*Not evaluable because of overlap with diploid proliferating population.
Chromosome 1 was found to be trisomic in two cells. The karyotype of clonal cells was 44, XY, −2, −3, −4, −6, −8, −10, −13, −14, −17, −18, −19, +2q+, der(3), t(3;6) (p11;p11), del(10) (q24), t(14;18) (q32;q21), +4mar (Fig 4B).

DISCUSSION

The conversion of non-Hodgkin’s lymphomas from “favorable” to “unfavorable” histologies is well-documented. Several recent clinical series estimate the risk of conversion to be from 15% to 40%, depending on how such risk is calculated, with even higher rates detectable at autopsy. Previous series have reported only a small number of cases with progression to diffuse undifferentiated lymphoma. In addition, Come et al described five cases of a “blastic” variant of N-PDLL with leukemic phase, three of whom eventually converted to histology with a “diffuse
growth pattern, an increased proportion of cells with blastic cytology, an increased mitotic index and prominent starry sky patterns." The intermediate transformation described in case 2 here, eight weeks prior to the Burkitt's-like transformation, may resemble the blastic variant they reported. Catovsky et al\textsuperscript{10} reported a single case of a follicular lymphocytic lymphoma that presented simultaneously with Burkitt's-like cells in ascites. Our report describes three cases of a Burkitt's-like lymphoma arising in patients with N-PDLL, with phenotypic, cytogenetic, and kinetic characterization of these tumors.

The appearance of Burkitt's cells is quite distinctive, being characterized by intermediate to large cell size, fine nuclear chromatin, prominent nucleoli, deeply basophilic cytoplasm that stains strongly with methyl green pyronine, and abundant cytoplasmic vacuoles. Burkitt's is usually identified as a B cell malignancy, expressing monoclonal surface immunoglobulin.\textsuperscript{5,11-13} However, we,\textsuperscript{14} as well as others,\textsuperscript{15} have reported that occasionally non-B cell lineages may express typical L3 morphology. The extremely rapid growth observed clinically is reflected by marked elevations of serum LDH, high S-phase values determined by \textsuperscript{3}H-thymidine labeling or DNA flow cytometry, and high cellular RNA content.\textsuperscript{16,17} In addition, certain chromosomal translocations are associated with Burkitt's lymphoma.\textsuperscript{18,19} The most common translocation is t(8;14), but t(2;8) and t(8;22) also occur.

In each of our cases, the diagnosis of a Burkitt's-like lymphoma was made initially on the presence of typical L3 cells in the marrow. Lymph node biopsy was performed only in case 1 and revealed features typical of Burkitt's lymphoma with residual areas of N-PDLL. Although from a morphologist's point of view, lymph node and marrow appearance remain the sine qua non for a diagnosis of Burkitt's, several other features of these cases, though not specific, support comparison to a Burkitt's-like lymphoma. The clinical course, once transformation had occurred, was that of a high-grade lymphoma. Despite initial brief responses to intensive chemotherapy, the disease progressed rapidly, and survivals from transformation were 1, 2½, and five months. Meningeal involvement developed in two patients; the third patient received prophylactic intrathecal methotrexate. Serum LDH, frequently markedly elevated in Burkitt's lymphoma, exceeded 400 U/L in each case. Also consistent with a Burkitt's-like lymphoma was the high percent of cells in S phase. In each case, at least 15% of cells were in S phase, values typical of only high-grade lymphomas (Burkitt's, some cases of diffuse histiocytic, and other undifferentiated lymphomas).\textsuperscript{16,17} In case 3, S phase had been previously documented to be only 3% prior to transformation, in the expected range for nodular lymphomas. In case 2, the high S-phase value detected prior to the appearance of Burkitt's-like cells likely reflected an intermediate stage of transformation, also reflected by high LDH levels and cytologic features. Values for RNA index, previously described by us\textsuperscript{17} as being low for low-grade and high for high-grade non-Hodgkin's lymphomas, were noted to increase significantly following transformation in each case.

In addition to the clinical, cytologic, and kinetic changes observed, evidence that the Burkitt's-like cells represented a distinct transformation from N-PDLL was provided by DNA flow cytometry. In each case, transformation in marrow was associated with a change in the DNA stemline. In cases 1 and 3, DNA stemline increased from 2.0 to 2.5 and 2.2, respectively—a change commonly associated with clonal evolution of malignancies; in case 2, DNA stemline decreased from 2.1 to 1.9. These changes are consistent with previous observations that higher grade malignant lymphomas are more likely to have abnormal DNA stemlines than low-grade lymphomas.\textsuperscript{17}

Surface marker analysis performed prior to transformation documented the B cell origin of each N-PDLL. Following transformation, cells from lymph node of case 1, which had been predominantly replaced by Burkitt's-like lymphoma, continued to express sIgG\textsubscript{\alpha}. In none of the cases, however, was sIg detectable on Burkitt's-like cells from bone marrow following transformation. The absence of sIg on bone marrow cells was somewhat surprising, as sIg was detected on lymph node cells in case 1 and it is presumed that the Burkitt's-like cells in all three cases arose from N-PDLL and would therefore have been expected to preserve the sIg(+) phenotype. Failure to demonstrate sIg could have been due to capping-off of sIg prior to analysis or to loss of expression following transformation. Discrepancy between sIg expression by Burkitt's-like cells from lymph node and marrow from case 1 could have indicated further evolution of cells in bone marrow. Consistent with this is the observation that the abnormal DNA stemline detected in marrow was not detected in lymph node. Finally, it is also conceivable, but considered less likely, that these cases represented the development of a second malignancy not derived from N-PDLL.

Metaphases following transformation were available for karyotype analysis in cases 1 and 2. Each contained a t(14;18), the most common translocation found in nodular lymphomas,\textsuperscript{20-23} and neither contained the t(8;14), commonly found in Burkitt's lymphoma.\textsuperscript{18} Unfortunately, karyotypes of original N-PDLL samples were not done. However, the finding of the t(14;18) in two of the cases is at least consistent
with the origin of the Burkitt's-like cells from N-PDLL, as t(14:18) is found in the majority of follicular lymphomas and has not been reported in Burkitt's lymphoma.

The concept of clonal evolution with progressive "dedifferentiation" of malignant cells is described in many malignancies. This phenomenon occurs not only in non-Hodgkin's lymphomas, but also may be seen in the progression of chronic lymphocytic leukemia to diffuse histiocytic lymphoma (Richter's syndrome), in the blast crisis of chronic myelogenous leukemia, and in nonhemopoietic malignancies, such as astrocytomas and soft tissue sarcomas, in which recurrences are commonly accompanied by increasing clinical and histologic aggressiveness. In some of these cases, evidence for clonal evolution from the original malignancy has been provided by surface marker analysis, with preservation of expression of monoclonal IgG, or by karyotyping. However, such evidence is available in only a limited number of cases, and it cannot be assumed that such "conversions" do not at least sometimes represent the development of second malignancies. Undifferentiated lymphomas have been reported to develop in patients previously treated for Hodgkin's disease. In one case presented here, evidence for clonal derivation was provided by preservation of monoclonal IgG in Burkitt's-like cells seen arising in lymph node. However, the lack of IgG detectable on marrow cells and the absence of available karyotypes prior to transformation leave this unproved.

This report extends the previously described phenomenon of conversion of favorable lymphomas to more aggressive patterns by describing three patients who developed clinical, morphological, and kinetic features of a Burkitt's-like lymphoma. Changes observed clinically and morphologically were accompanied by abnormalities in serum LDH, S-phase values, DNA stemline, and RNA content. Despite aggressive therapy, which has proven to be increasingly effective in some cases of primary Burkitt's lymphoma, remissions in the present cases were brief and survival was short. Whether this relates to resistance induced by extensive prior chemotherapy and the development of a new clone of neoplastic cells on a background of prior drug-induced immunosuppression is unknown.

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